

TEXAS MEDICAL CENTER NASA/JOHNSON SPACE CENTER

COOPERATIVE AGREEMENT PROGRAM NCC 9-36, ROUND II

**COVER SHEET FOR FINAL REPORT**

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Countermeasures to Bone Loss

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## Applications of Anabolic Vitamin D Analogs as Countermeasures to Bone Loss

The experiments in Round 2 were designed to extend the results of our efforts in Round 1 which led us to hypothesize that the seco-steroid, 1,25-dihydroxyvitamin D<sub>3</sub> [1,25(OH)<sub>2</sub>D<sub>3</sub>], acts in synergy with parathyroid hormone (PTH) to regulate bone calcium homeostasis<sup>1</sup>. Our work centered on one particular target of 1,25(OH)<sub>2</sub>D<sub>3</sub> action, the voltage-sensitive calcium channels (VSCC's), which are activated acutely by this steroid within milliseconds of exposure<sup>2</sup>.

We found that the vitamin D analog, AT, which cannot occupy nuclear vitamin D receptors, was also a potent agonist of PTH priming. In these two months we have learned that two other steroids, estrogen and progesterone, are ineffective as priming agents [although estrogen at high (nonphysiological) concentrations will prime bone cells' response to PTH]. Interestingly, our data indicated that the 24,25-dihydroxy metabolite of vitamin D<sub>3</sub> can neither cause intracellular Ca<sup>2+</sup> transients nor modify the response of bone cells to PTH. This exquisite specificity of 1,25(OH)<sub>2</sub>D<sub>3</sub> suggests an important new role for this hormone in the regulation of bone mass.

We also determined that Ca<sup>2+</sup> channel expression (mRNA level) is reduced in cultured rat calvarial cells treated with the synthetic steroid, dexamethasone, compared to untreated control cells. When cells treated with dexamethasone were loaded for 1, 3, and 5 weeks, respectively, there was up regulation of the mRNA that was dependent on the duration of loading. This is further evidence of steroid-linked regulation of VSCC expression in bone cells, and supports our interpretation of the results of our experiments with vitamin D<sub>3</sub>.

A second area of research focused on the effects of mechanical strain on VSCC expression in bone. These experiments were performed in collaboration with Dr. Steven Goldstein (Univ. Michigan), who generously provided RNA extracted from dog bones that had been exposed to mechanical strain *in vivo*. Our results suggest that mechanical loading elevated VSCC expression in the long bones from 3 of the 6 animals tested.

To confirm the identity of the VSCC isoform expressed in dog bone, we used a set of PCR primers designed for rat calcium channel on dog RNA samples. The primers amplified 246 base pair (bp) fragment from cDNA synthesized using as a template mRNA from ROS cells, primary rat calvarial cells and rat bone marrow cell culture. Similarly, this set of primers also amplified a 246bp DNA from dog bone cDNA samples. In addition to the 246bp PCR product, we consistently see another 600bp product in dog samples. These data suggest a unique form of Ca<sup>2+</sup> channel in dog bone; we hypothesize that this form may be present in human bone as well.

A second line of experimentation, carried out in collaboration with Dr. Randall Duncan, a NASA-funded investigator in Indianapolis, centered on RT-PCR analysis of effects of mechanical strain on Ca<sup>2+</sup> channel expression in cultured bone cells. Compared to unstrained controls, the expression of vitamin-D-sensitive Ca<sup>2+</sup> channels is elevated 3- to 5-fold over a 24 hr period.

One presentation of data generated by Round 2 research was made<sup>3</sup>, and two abstracts have been submitted.

## References:

1. Li, W., Duncan, R.L., Karin, N.J. and Farach-Carson, M.C. (1997). 1,25 (OH)<sub>2</sub>D<sub>3</sub> enhances PTH-induced Ca<sup>2+</sup> transients in preosteoblasts by activating L-type Ca<sup>2+</sup> channels. *Am. J. Physiol.* 273:E599-605.
2. Caffrey, J.M. and Farach-Carson, M.C. (1989). Vitamin D<sub>3</sub> metabolites modulate dihydropyridine-sensitive calcium currents in clonal rat osteosarcoma cells. *J. Biol. Chem.* 264:20265-74.
3. Li, W., Duncan, R.L., Karin, N.J. and Farach-Carson, M.C. (1997). 1,25(OH)<sub>2</sub>D<sub>3</sub> Enhances Parathyroid Hormone-Induced Ca<sup>2+</sup> Transients in Pre-Osteoblasts by Activating L-Type Ca<sup>2+</sup> Channels. Tenth Workshop on Vitamin D, Strasbourg, France.